

Experimental results: Shelf-life studies of different PS products

The shelf-life studies followed the guidelines of the International Committee for Harmonization (ICH). More specifically, these studies followed the ICH harmonized tripartite guideline for "Stability testing of new drug substances and products", Q1A(R2), recommended for adoption at step 4 of the ICH process on 6 February 2003 by the ICH Steering Committee. These tests included both standard (ambient) conditions (Room Temperature) and accelerated or stress conditions (35°C, 65% Relative Humidity)

1. Stability of non-stabilized conventional fluid PS capsules

The level of non-stabilized fluid PS was measured, when encapsulated under room temperature and accelerated conditions.

The following figure demonstrates that standard fluid PS may degrade by 10% over time, following approximately 3 months (12 weeks) after encapsulation. Moreover, it has been shown that accelerated conditions may indicate PS degradation even within 4 weeks following encapsulation and that the ratio between room temperature and accelerated conditions is approximately 1:4.

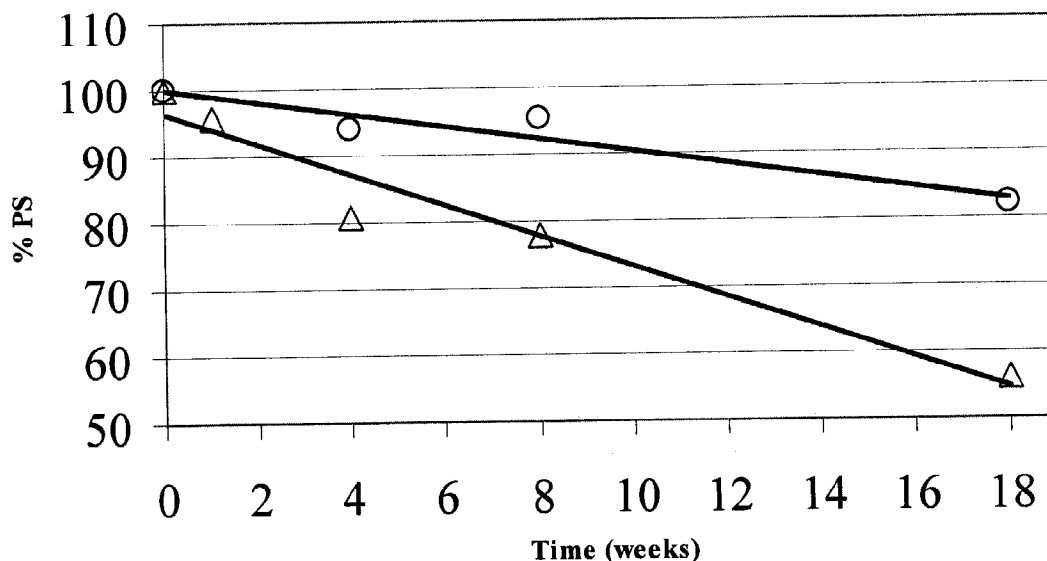


Figure 1: ^{31}P -NMR analysis results for 100mg "standard" fluid PS-containing soft-gel capsules at RT (O) and accelerated (Δ) storage conditions over 18 weeks, calculated as percentage of PS.

Raw data is provided in Table 1.

Table 1: ^{31}P -NMR analysis results for non stabilized fluid PS-containing soft-gel capsules at RT and accelerated storage conditions over 18 weeks.

Time	NMR at RT	NMR at accelerated conditions
T=0	100.0%	100.0%
week 4	93.9%	80.6%
week 8	95.3%	77.8%
week 18	81.9%	55.8%

2. Long-term Stability study of dispersion capsules containing 73 mg PS/capsule

The initial level of PS in the capsules used in the initial long term study was 75 mg, analyzed by ^{31}P -NMR to be approx. 73 mg/capsule at baseline, immediately after softgel capsule production. As described above, production of these capsules aimed at a minimal level of 75mg PS per capsule. As can be seen in Figure 2, this level fluctuates around this figure for 1 year from production, at either RT or accelerated conditions, and as expected, 2 years after encapsulation no change in PS level was measured under RT. Fluctuation in PS levels, appearing as an increase in PS levels, is within the standard error of the method and may be correlated with changes of NMR conditions and calibration in light of the long periods of time between each sample analysis.

Note should be taken of the fact that the PS content in capsules kept at ambient conditions is practically identical to the PS content in capsules kept at accelerated conditions. The excellent results obtained at accelerated conditions point to the fact that the shelf life of softgel capsules containing stabilized PS preparation can be easily extrapolated to be at least up to 4 years, based on applicant's previous studies, which suggest 1:4 time ratio.

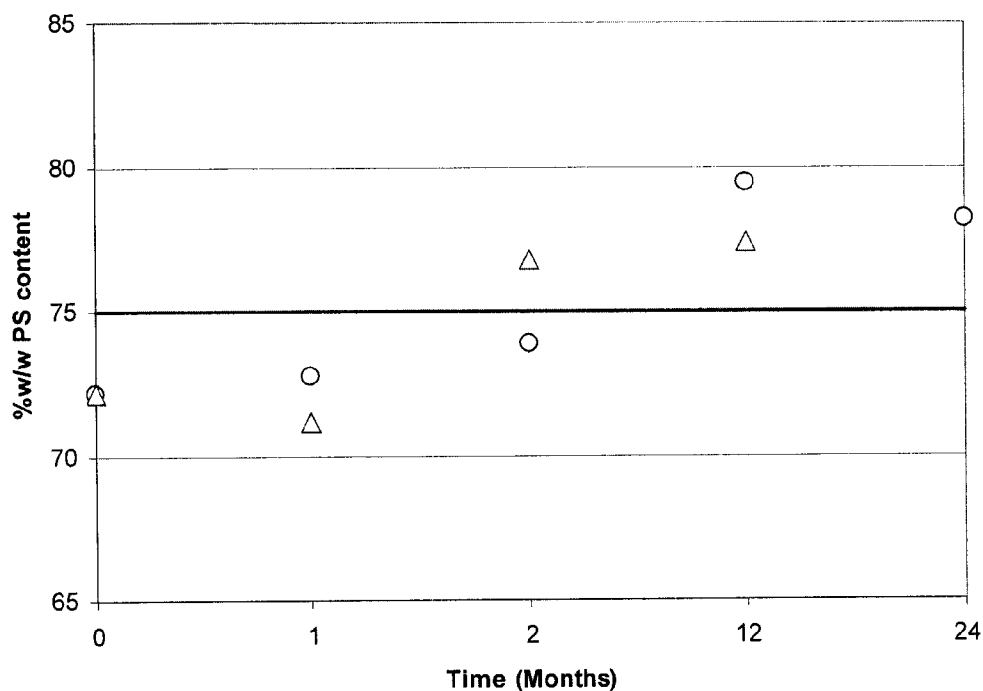


Figure 2: ^{31}P -NMR analysis results for 75mg stabilized dispersion soft-gel capsules at RT (O) and accelerated (Δ) storage conditions over 2 years.

Raw data is provided in Table 2.

Table 2: ^{31}P -NMR analysis results for 75mg of PS in stabilized PS dispersion soft-gel capsules at RT and accelerated storage conditions over 2 years.

Time (Months)	NMR at RT	NMR at accelerated conditions
T=0	72.2	72.2
1	72.7	71.1
2	73.9	76.8
12	79.4	77.4
24	78.2	NA

Total average is 74.9 ± 3

3. Long-term stability study of new batch dispersion capsules containing 105 mg PS/capsule

A second shelf-life study using a new batch of softgel capsules, designed to contain 105mg of PS each. The initial level of PS in the capsules used in the initial long term study was found by ^{31}P -NMR to be 107mg/capsule at baseline, immediately after soft-gel capsule production. As can be seen in Figure 3, the level of PS fluctuates around the 105 mg target and has not dropped below the 100mg/capsule throughout the duration of the study. No decrease in PS content or PS degradation can be observed during this period.

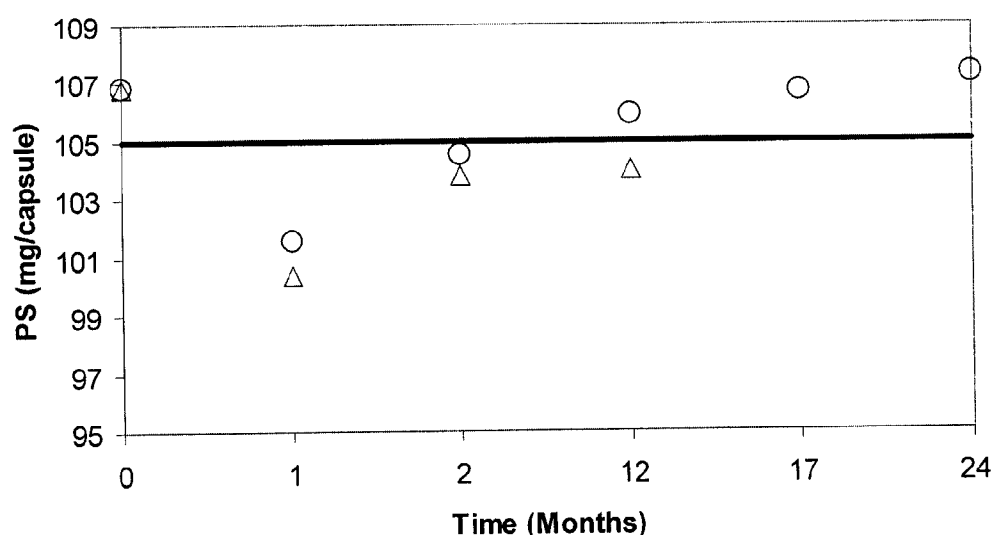


Figure 3: ^{31}P -NMR analysis results for 105mg of stabilized PS-containing soft-gel capsules at RT (○) and accelerated (△) storage conditions over 12 months.

Raw data is provided in Table 3.

Table 3: ^{31}P -NMR analysis results for 105mg of stabilized PS-containing soft-gel capsules at RT and accelerated storage conditions over 1 year.

Time (Months)	NMR at RT	NMR at accelerated conditions
T=0	106.8	106.8
1	101.6	100.4
2	104.5	103.8
12	105.9	104.0
17	106.7	-----
24	107.3	-----

Total average is 104 \pm 2

As in the case of the long-term study conducted on the 75mg capsules, the PS content in capsules kept at ambient conditions is practically identical to the PS content in capsules kept at accelerated conditions. These excellent results obtained at accelerated conditions, even for this limited study period, indicate a repetition of the excellent shelf-life results obtained at the initial study and to the fact that even at higher PS levels, such as 100mg/capsule, the shelf-life of the stabilized PS-containing capsules is superior and no decrease in PS levels is expected for at least 4 years.

4. Other Comparative results

Some attention has been given to other commercially available products which are supplied under the guarantee of extended shelf life. As seen in Figure 4, commercially available soft gels were tested under accelerated conditions and compared to the stabilized PS dispersion (assigned as 20D technology) of the invention.

As clearly shown by this study, PS by the other supplier (Supplier A), claimed to be storage-stable, failed to achieve the stability of the stabilized PS preparation of the invention. Standard PS that appears in Figure 4 relates to the non stabilized phosphatidylserine.

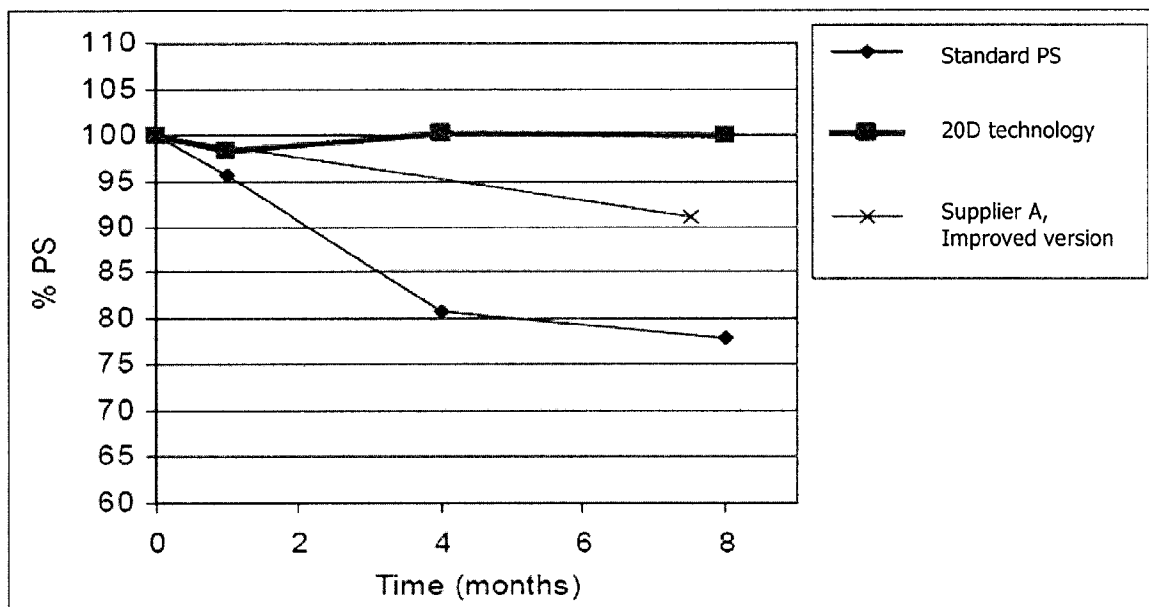


Figure 4: A comparative ^{31}P -NMR analysis results between various material sources, under accelerated conditions is presented in this study. Conventional approach to more harsh conditions does not resolve stability issues but slightly slows it down.

5. Stability of powdered PS

Stabilized powder PS was prepared according to the invention. The PS concentration was 70% (w/w). Stability tests were performed at ambient temperature and included two parameters: peroxide value (PV) measurements and %PS measured by HPTLC internal-method against a standard that contain 70% PS (standartized by NMR analysis) and kept under -40°C temperature. Results are summarized in Table 4:

Table 4: Stability data of stabilized powder PS preparation containing 70% PS

Time (months)	Peroxide value	%PS*
0	<0.5	100%
11	<0.5	102%
15	1.05	101.8
21	<0.5	100.8

*Against standard that contains 70% phosphatidylserine

As can be seen from Table 4, the stabilized PS remained stable toward oxidation and decomposition for at least 21 months.